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# Compatibility of the Aphid Fungus *Cephalosporium lecanii* with the Leafminer Parasite, *Diglyphus beginii* (Hymenoptera: Eulophidae)

JAMES A. BETHKE AND MICHAEL P. PARRELLA

Department of Entomology, University of California, Riverside, California 92521.

*Abstract.*—The effect of the aphid-specific fungus, *Cephalosporium lecanii,* on three life history parameters of the leafminer parasite, *Diglyphus begini* (Ashmead) were investigated. When treated directly with the fungus ($1.4 \times 10^6$ colony forming units/g) or exposed to chrysanthemum leaf surfaces treated with the fungus, no effect on parasite longevity was found. However, *D. begini* adults held on close confinement with fungal infected aphids (*Aphis gossypii* Glover) had significantly reduced longevity when contrasted with controls. The presence of fungus-infected aphids on whole chrysanthemum plants did not affect pre-oviposition period, fertility or longevity of the *D. begini* parent generation or of their offspring.

Fifteen species of aphids are reported to attack chrysanthemums, *Dendranthema grandiflora* (Tzevel) worldwide (Blackman and Eastop, 1985) with four dominating as major pests: *Aphis gossypii* Glover, *Mysus persicae* Sulzer, *Brachycaudus helichrysi* Kaltenbach and *Macrosiphon sanborni* Gillette. In the development of an integrated pest management (IPM) program for chrysanthemums, management strategies for aphids must be developed and these should be compatible with control strategies for other chrysanthemum pests. In England, two aphid control strategies are recommended: use of the selective aphicide pirimicarb and use of the aphid-specific fungus *Cephalosporium lecanii* (Zimm.) (Wardlow, 1985). In the United States, use of pirimicarb was banned in the late 1970's, which greatly reduced the IPM options for aphid control. *Cephalosporium lecanii,* while never registered in the United States, offers good potential for aphid control especially in chrysanthemum production where environmental conditions necessary for the development of epizootics are often met (Hall, 1981; Parrella, 1987).

Although Hall (1985) indicated that *C. lecanii* was compatible with leafminer parasites, no data were reported. In addition, the fungus has been shown to cause mortality in the aphid parasite *Aphidius matricariae* Hal. (Scopes, 1970) and in the whitefly parasite *Encarsia formosa* Gahan (Ekbom, 1979). At present, releases of the leafminer parasite *Diglyphus begini* Ashmead are suggested for control of *Liriomyza trifolii* (Burgess) on chrysanthemums in the U.S. (Parrella and Jones, 1987). The leafminer is the major pest on this crop and any pesticides used for control of other chrysanthemum pest (e.g., aphids) must be compatible with *D. begini.* Ideally, the pesticide should have no effect on adult parasites via direct topical application or contact with residues and it should not effect developing, immature stages of the parasite. We evaluated the compatibility of *C. lecanii* with

*D. begini* by determining the direct effect of the fungus on the preoviposition period, longevity and fertility of the parasite. The possible influence of *C. lecanii* on the offspring ($F_1$ generation) of exposed parasites was also determined.

## Materials and Methods

Vertalec®, a commercial formulation of *C. lecanii* (Tate & Lyle Ltd., England) was used as the fungal source. An estimate of spore viability was obtained by serially diluting the recommended rate of Vertalec® (2.5 g/liter water), plating the dilutions on potato dextrose agar (PDA), then counting the number of colonies produced. The effect of *C. lecanii* on the life history parameters mentioned earlier was examined in 2 ways: adults were placed on individual leaves in Munger cells and adults were caged with whole chrysanthemum plants in an environmental chamber.

*Munger cell study.*—Five 1–2-day-old adult *D. begini* selected from a greenhouse colony (Parrella et al., 1989) were confined in a modified Munger cell (Munger, 1942). Parasites were subjected to one of the following treatments: 1) parasites sprayed with water in a Kearns-March knockdown chamber (Kearns and March, 1943) (5 sec at 10 psi) and placed on an untreated chrysanthemum leaf, 2) parasites treated with *C. lecanii* at the recommended rate (2.5 g/liter water) in a Kearns-March knockdown chamber (5 sec at 10 psi) and then placed on an untreated chrysanthemum leaf, 3) untreated parasites on a leaf treated with the recommended rate of *C. lecanii,* and 4) untreated parasites on a leaf containing >10 aphids (*A. gossypii*) infected with *C. lecanii.* These aphids were infected by applications of the recommended rate of *C. lecanii* in a 133-$m^2$ chrysanthemum greenhouse. Advanced infection was evident in these aphids (hyphae present). Each treatment was replicated four times (4 reps., 5 parasites/rep.). A honey and water solution (2:1) was present as a food source in the Munger cells throughout the experiment.

Observations of mortality were recorded daily. In addition, parasites which died apparently from *C. lecanii* (hyphae growing from parasite) were placed on potato dextrose agar and compared to colonies plated as mentioned above.

*Cage study.*—Six hundred rooted chrysanthemums (var. 'white hurricane') were individually potted (10 × 10 × 10-cm pots) on 5 February 1987 in a 133-$m^2$ plastic greenhouse. Plants were grown at 24–27°C under supplementary lighting (75-W bulbs) to inhibit flowering. Humidity within the greenhouse was supplemented by a MEE II® (MEE Industries Inc., San Gabriel, California) cloudmaker. Temperature (described previously) and humidity (>80% RH) within the greenhouse were within the range considered optimal by Hall (1985) for infection by *C. lecanii.*

Many plants within the greenhouse were lightly infested with aphids (*A. gossypii*) at the time of treatment. However, before treatment, 200 aphid free plants were transported to another greenhouse to be used as control plants. One application of *C. lecanii* at the recommended rate was applied using a B & G hand sprayer to run off on 3 March 1987.

Plants were pinched back to five leaves to enable cages to be placed over them. Aphids were generally on the adaxial leaf surfaces and heavy infection was noted; a count of the aphids was not taken. The first infected aphid was observed on 13 March 1987 (10 days after application) and on 16 March 1987, 15 plants from the treated and untreated groups were exposed daily to separate colonies of *L.*

Table 1. Longevity of *Diglyphus begini* confined in Munger cells on chrysanthemum leaves under the following conditions: 1) leaves water sprayed, parasites untreated; 2) leaves water sprayed, parasites treated with *Cephalosporium lecanii*; 3) leaves treated with *C. lecanii*, parasites untreated; and 4) leaves containing aphids infected with *C. lecanii*.

| Treatment | | |
|---|---|---|
| Leaf | Parasite | Longevity (days) |
| Untreated | Untreated | 7.65 ± 0.89 |
| Untreated | Sprayed with *C. lecanii* | 9.57 ± 0.76 NS[1] |
| Sprayed with *C. lecanii* | Untreated | 7.52 ± 0.67 NS |
| Aphids infected with *C. lecanii* present | Untreated | 4.15 ± 0.20* |

[1] Means compared to control using *t*-test ($P = 0.05$, * $P = 0.0001$).

*trifolii* and then kept in different environmental chambers at 21°C, 14:10 L:D. Separate leafminer colonies and chambers were used to avoid transmission of the pathogen to untreated plants. In addition, treated plants were held in a chamber which set relative humidity at 90% to further promote infection.

Three to 4 days post-leafminer exposure, a pretreatment count of leafminer larvae within leaves was taken. Five to 6 days post-leafminer exposure, when leafminer larvae were large (probably third instars), 10 individual plants per treatment were covered with an inverted translucent plastic container (1 liter) in which the bottom was removed and replaced with screening for ventilation. One newly-emerged male and female *D. begini* were taken from a greenhouse colony and placed with each plant and the plant and parasites were covered with a cage. Cages of both treatments were held in an environmental chamber at 27°C, 14:10 L:D and parasites were given new plants of the same treatment on a daily basis; dead males were replaced when necessary. After a 24-hr exposure to the parasites, plants were cut at the soil line, held in quart size paper cartons (9-cm diameter × 17-cm depth) fitted with a glass vial at the top. Emerging leafminers and parasites moved into the vial and could be easily counted; these were removed daily. To evaluate possible fungal effects on subsequent generations, studies of possible sublethal effects on the progeny from both treatment groups were conducted in the same manner as previously described with the exception that these $F_1$ parasites were all given clean control plants (no aphids) containing leafminer larvae. One day's production of parasites from the parent generation was collected for use in this study. From this emergence, 10 parasites were randomly chosen and pre-oviposition, longevity and fertility of these parasites were recorded. Statistical analysis was performed using a *t*-test; each variable was compared to the control.

## Results

When plated on PDA, activity of the recommended rate of Vertalec® (2.5 g/liter water) was found to be $1.4 \times 10^6$ colony forming units (CFU) per gram. Gardner et al. (1984) using Vertalec® at 15 g/liter water found activity to be $10^8$ CFU/g.

*Munger cell study.*—The longevity of *D. begini* where the parasites and chrysanthemum leaves were treated with water only was 7.65 days (Table 1). Those treatments where the chrysanthemum leaf or parasite were sprayed with *C. lecanii*

Table 2. Longevity and fertility of *Diglyphus begini* when exposed to untreated plants or plants infested with aphids infected with *Cephalosporium lecanii* and their progeny when exposed to untreated plants. Plants were previously exposed to leafminers to insure that leafminer larvae were present for the parasites.

| Parent generation | | | |
|---|---|---|---|
| Plants | *n* | Longevity[1] (days) | $\bar{x}$ no. of adult *D. begini* produced per day[1] |
| Untreated | 10 | 8.4 ± 1.1 | 1.7 ± 0.3 |
| | | NS | NS |
| Plants with aphids infected with *C. lecanii* | 10 | 5.2 ± 1.1 | 2.7 ± 0.6 |
| **$F_1$ generation** | | | |
| Source of $F_1$ progeny | *n* | Longevity[1] (days) | $\bar{x}$ no. of adult *D. begini* produced per day[1] |
| From untreated group[2] | 6 | 8.7 ± 3.6 | 5.6 ± 0.9 |
| | | NS | NS |
| From group exposed to aphids infected with *C. lecanii*[2] | 9 | 8.1 ± 2.0 | 4.4 ± 0.6 |

[1] Means are not significantly different (NS) using a *t*-test ($P = 0.05$).
[2] $F_1$ generation were given untreated plants free of aphids.

were not significantly different from the control. However, those exposed to *A. gossypii* with advanced fungal infection had a significantly shorter longevity (ca. 4.0 days). Dead parasites placed on PDA agar, initiated colony formation of *C. lecanii*.

*Cage study.*—The preoviposition period of *D. begini* was ca. 2.3 days for treated and untreated groups; no significant differences were found (*t*-test, $P = 0.05$). In addition, no differences were found in the longevity or fertility for the parent or $F_1$ generation of *D. begini* exposed to either treatment (Table 2). However, more young were produced per day by the progeny of both parental groups; this was not observed in the caged study (below).

Both the parent and $F_1$ generation *D. begini* successfully attacked *L. trifolii* in the caged study (Table 3). Mean percent leafminer mortality per day in the parent generation under the infected aphid treatment was significantly greater compared to *D. begini* in the untreated control; however, this may have been caused by higher numbers of leafminer larvae in plants used in the infected aphid treatment. The $F_1$ generation of either untreated or fungal treated *D. begini* did not differ in their ability to kill leafminer larvae (Table 3). However, overall percent mortality of leafminers was greater for the $F_1$ generation than for the parent generation.

## Discussion

*C. lecanii* is a pathogenic fungus which has been separated into strains via spore size; a large one which is specific to aphids and a smaller one which attacks whiteflies (Samson and Rombach, 1985). Pathogenic fungi are potentially one of the most versatile pathogens available in glasshouses. They usually infect large host ranges, different stages and ages of host, need not be ingested to become infective, are virulent and disperse naturally (Fuxa, 1987). A disadvantage is that an initial period of high humidity is required for the germination of the applied spores. However, glasshouse chrysanthemum production is particularly suited for

Table 3. Mortality of *Liriomyza trifolii* due to *Diglyphus begini* during cages study. Mortality includes natural mortality plus parasite-induced mortality.

| Parent generation<br>Plants | $\bar{x}$ no. of leafminer larvae per plant[1] | $\bar{x}$ no. of leafminers produced per day[1] | $\bar{x}$ percent mortality of leafminers per day[1] |
|---|---|---|---|
| Untreated | 10.7 ± 0.5 | 6.2 ± 0.6 | 0.44 ± 0.04 |
| | * | NS | * |
| Plants with aphids infected with *C. lecanii* | 13.7 ± 1.0 | 4.8 ± 0.8 | 0.62 ± 0.04 |
| **$F_1$ generation**<br>**Source of $F_1$ progeny** | **$\bar{x}$ no. of leafminer larvae per plant[1]** | **$\bar{x}$ no. of leafminers produced per day[1]** | **$\bar{x}$ percent mortality of leafminers per day[1]** |
| From untreated group[2] | 15.8 ± 1.2 | 2.4 ± 0.5 | 0.83 ± 0.04 |
| | NS | NS | NS |
| From group exposed to aphids infected with *C. lecanii*[2] | 14.9 ± 1.0 | 4.2 ± 0.8 | 0.73 ± 0.04 |

[1] * = significantly different using a *t*-test ($P = 0.05$); NS = not significantly different using a *t*-test ($P = 0.05$).

[2] Progeny were given untreated plants free of aphids.

fungal infection. Normal production practices include covering the crop with black polyethylene to restrict day length and stimulate flowering. This depends on the latitude, and generally occurs in California from April to September.

Hall and Burgess (1979) reported *Myzus persicae* (Sulzer) is not inherently less susceptible to *C. lecanii* than *Macrosiphum sanborni* (Gillette) due to ecological or behavioral factors. *Diglyphus begini* is also not susceptible, probably due to similar reasons. *Diglyphus begini* usually searches the abaxial surface of chrysanthemum leaves since *L. trifolii* mines the upper mesophyll layer (Parrella et al., 1985). Infected aphids are usually on the adaxial surface of leaves or at the terminal.

Parasites confined to Munger cells with infected aphids survived for a shorter time than those in the caged study. Several possible explanations for this are: 1) they could readily contact fungal spores and hyphae, as they were exposed to undersides of leaves with aphids and hyphae present, 2) spores from the aphids may be more infectious than sprayed formulation [however, Hall (1981) stated that this was not the case with *C. lecanii*], and 3) there simply may be greater numbers of active spores available.

Gardner et al. (1984) suggests Vertalec® could prove to be an effective and economical alternative to chemical insecticides for aphid control. Since *C. lecanii* had a negligible effect on *D. begini,* it could be used in chrysanthemum production for control of aphids where this parasite is being used for leafminer control. Hall (1981) stated that *C. lecanii* could not be used prophylactically because of short spore longevity; however, Helyer and Wardlow (1987) demonstrated good control of *A. gossypii* and *M. persicae* on chrysanthemum with frequent low dose applications of Vertalec, and found this method to be compatible with potentially harmful fungicides.

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